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Bioaccumulation and depuration of zinc in the tissues of *Heterobranchus longifilis* exposed to zinc oxide nano-particles

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Abstract

Industries use zinc oxide nano-particles (ZnO-NPs) to produce most commercial and medicinal goods, but indiscriminate discharge of their effluents into the aquatic environment may accumulate in the tissues of the fish. The ability of these metal oxides to accumulate in the tissue of fish due to their nano size could lead to a reduction in the nutritional value of the fish. Therefore, this study aimed to evaluate the rate of zinc bioaccumulation in tissues of *Heterobranchus longifilis.* Juveniles of *H. longifilis* were exposed to sub-lethal concentrations (0.0, 6.00, 8.00, 10.00, 12.00 mg/l) of ZnO-NPs for 60 d and depurated for 30 d. At the end of each experiment, fish were sacrificed and analyzed for bioaccumulation. The increasing order of concentration of zinc was as follow: bone< muscle < blood < skin < kidney < liver < GIT < gills having 0.068mg/g to 0.263 mg/g from muscle to gills. That was above the permissible limits (0.050 mg/g) in fish tissues, except in the bone with 0.054 mg/g, which was still within the acceptable limits. Gills accumulated the highest (0.263 mg/g) content of zinc, and bone had the lowest (0.054 mg/g). After the depuration period, the content of zinc in the tissues decreased in all the tissues but still remained above the maximum permissible limits in gills, GIT, kidney, liver and skin (0.175 mg/g, 0.162 mg/g, 0.066 mg/g, 0.071 mg/g and 0.073 mg/g respectively). However, the bone, blood and muscle were able to depurate the zinc content to values (0.031 mg/g, 0.034 mg/g, and 0.044 mg/g, respectively) below the acceptable limits of Zn in fish tissues. The results indicated that zinc from ZnO-NPs bioaccumulated in *H. longifilis tissues*, but the fish could depurate the metal naturally. However, the ability to depurate the bioaccumulated metal is time-dependent.

Keywords: Bioaccumulation, Depuration, *Heterobranchus longifilis*, Zinc oxide nano-particles, Tissues

Introduction

The rise in population growth, urbanization, industrialization and other human activities has greatly led to a decline in fish population due to an increase in chemical pollution. Among the chemicals pollution that causes great decline in fish population are the emergent nano-particles like zinc oxide nano-particles. Zinc oxide nanoparticle is a white powder that is insoluble in water and is one of the inorganic compounds of group II–IV semiconductor for analytical sensing applications (Matinise *et al*., 2017). The small size and the large space of ZnO-NPs enable it to penetrate and accumulate within cells which causes damage to the cell membrane through the reactive oxygen species (ROS) mechanism (Yang *et al*.,

2009). Zinc oxide nano-particles (ZnO-NPs) are typical metal oxide nano-particles that are widely used in a range of products, such as medicines, cosmetic, textile, paints and in the treatment of waste water because of their low biocompatibility and biodegradability (Zimmermann *et al*., 2012). Despite these benefits, the increase in the use of nanoparticles and indiscriminate discharge of their effluents from the industries into the aquatic environment has become a concern of scientists on the potential hazard effects of nanomaterials on man and environmental health (Blaise *et al.*, 2008). Toxicity of most nano-particles is in relation to the dissolved metal ions, when ZnO-NPs are dissolved in water, they release free zinc ions which are the primary source of toxicity (Blinova *et al*., 2010; Buerki-Thurnherr *et al*., 2013).

ZnO-NPs toxicity is therefore related to their large surface areas, released Zn ion and surface charges which are reported to initiate contact between the nano materials and cells. (Adams *et al*., 2006 and Aruoja *et al*., 2009). The ability of metal oxide nano-particles to persist and bioaccumulate in tissues could cause damage in the tissues thereby interrupting the functions of affected tissues. This could lead to depletion of oxygen and reduction in respiration rate thereby inducing hyperactivity and oxidative stress. Occurrence of these particles on the water bodies may cause ecological changes and degradation of natural spawning ground in water bodies. This greatly affects fish production thereby reducing diversity of fish species. Fish are used in ecotoxicological assessment because of their link in food chain. *Heterobranchus longifilis* is a fast-growing fish that is endemic to Africa and constitutes one of the major animal proteins that is well accepted by consumers because of their availability, palatability and health provision (Fawole *et al*., 2007). The steady growth of fish farming in Nigeria has greatly improved aquaculture industry and the national economy as a whole. However, the lives of most of these fish species are endangered as a result of various environmental contaminations which may lead to disappearance of this commercially important fish species (Ademoroti, 1996). The quality of the survived ones may be affected due to accumulation of these metal oxides in their tissues, and this could lead to reduction in the nutritional value of the fish. The potential hazards of the contamination of water bodies on fish and human health deserve greater attention. Several studies have been carried out on the accumulation of various nano-metals in different parts of fish tissues such as the gill, liver and intestine of common carp after 30 days of exposure to ZnO-NPs (Wang *et al*., 2011; Hao *et al*., 2013; Linhua *et al.,* 2013). There have been other studies in goldfish (*Carassius auratus*) by Johnston *et al*. (2010), Uystal (2011) and Fan *et al.* (2013). However, there is paucity of information on ZnO-NPs and none so far on the effect of this metal oxide nano-particles on *Heterobranchus longifilis*. There is therefore the need to investigate the rate of accumulation of ZnO-NPs in different tissues of *Heterobranchus longifilis* which has a high value of protein, carbohydrate, fats and oil, minerals, fatty acids, amino acids and vitamins. This study therefore evaluated the bioaccumulation of Zn in the tissues of *H. longifilis* exposed to ZnO-NPs.

Materials and Methods

Experimental setup

Juveniles of *Heterobranchus longifilis* (average weight 19.40 ± 3.65 g and average length 12.45 ± 0.13 cm) were obtained from a commercial fish hatchery in Lagos. They were transported in plastic aquaria containing water from the hatchery to the of Zoology laboratory, Faculty of Life Sciences, University of Ilorin. The fish were not fed throughout the but commenced the following day to avoid indigestion and mortality. They were fed twice daily at 9.00 am and 5.00 pm with commercial feeds (Copens 2 mm) at 4% of initial body weight, following the procedure of Meyers *et al*. (1993). The fish were kept in large tank of 1000 litre capacity at 24^{o} C and acclimatized to laboratory conditions for 14 days prior to experiments. The water was kept oxygen saturated with aerators. Unconsumed feed and faecal wastes were removed and renewed every 24 hours to maintain the toxicant's concentration and to prevent pollution (FAO, 1986). Feeding was stopped 24 hours before the commencement of the experiment in line with (USEPA) 1996 protocol and water quality parameters were continuously monitored for temperature, pH, dissolved oxygen (D.O.), biochemical oxygen demand (BOD), chemical oxygen demand (COD), and conductivity according to APHA (1995) procedure. The powdery form of Sigma Aldrich (USA) as characterised by Akanbi-Gada (2019) was used for the experiment.

A stock solution of ZnO-NPs was prepared by dispersing zinc oxide powder into distilled water and the suspending solution of ZnO-NPs was stirred rigorously with magnetic stirrer for about two hours to break the precipitates. Each aquarium containing the toxicant was renewed every 24 h to maintain the toxicity in line with (FAO) 1986 procedure. Based on the result of the acute tests, the test fishes were

exposed to varying concentrations (0.00, 6.00, 8.00, 10.00 and 12.00 mg/l) measured from the stock solution of ZnO-NPs for 60 days. The fish were randomly grouped into six groups of twenty fish each. One group served as control without toxicant. After the exposure period, five fish from each of the experimental and control groups were randomly selected and sacrificed to remove the organs and blood was collected to estimate the rate of zinc bioaccumulation in the tissues.

Ethical approval

Ethical clearance fot this study was obtained from the University Ethical Review Committee (UERC) of the University of Ilorin, Ilorin, Nigeria, with approval number UERC/ASN/2016/648.

Depuration experiment

The fish from different concentrations were removed from the experimental aquaria, put into new aquaria containing fresh water only, and fed twice daily for 30 days. At the end of the depuration period, the fish were sacrificed after 15 and 30 days, blood and tissues were collected for determination of zinc bioaccumulation.

Bioaccumulation of ZnO-NPs

Tissue samples including blood, bone, gastro intestinal tract, gill, kidney, liver, muscle, and skin were collected. They were rinsed, dried, grinded, weighed and subjected to atomic absorption spectrophotometer (AAS) to determine zinc accumulation and calculate bio concentration factor (BCF). The tissues were then put into Kjeldahl flask for digestion following the methods adopted by Yi *et al*. (2011) and Ali *et al*. (2016). The digested tissues were diluted with de-ionized water and zinc concentration was then analyzed for Zinc concentration using Perkin-Elmer (Model 2380) atomic absorption spectrophotometer.

Bio concentration factor (BCF)

The bio concentration factor was evaluated using the following expressions:

$$BCF = \frac{\text{Concentration of Zn in tissue}}{\text{Concentration of Zn in water}}$$

To convert the concentration of zinc in mg/l to mg/kg

$$X \text{ mg/kg} = \frac{\text{Concentration (mg/l)} \times \text{volume of digested sample}}{\text{Gram of sample digested}}$$

$$BCF = \frac{X \text{ mg/kg}}{\text{Concentration of zinc in water}}$$

Data analyses

The data obtained from the study were expressed as mean ± standard deviation (S.D.). To assess the significant differences, one way analysis of variance (ANOVA) was used, followed by Duncan's Multiple Range Test (DMRT) (Duncan, 1955). The data were analyzed using SPSS 16.0 version. Statistical significance for all the tests was set at 95% ($P < 0.05$).

Results

The results obtained from the study revealed significant increase ($P < 0.05$) in the of total dissolved solid (TDS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), conductivity and turbidity in the groups exposed to ZnO-NPs, with a decrease in the dissolved oxygen (D.O.), except for temperature and pH with no significant difference ($P > 0.05$) compared to control (Table 1). However, a gradual decrease in the values of COD, conductivity and turbidity with increase in that of DO was recorded during the depuration period of 30 days (Table 1).

The zinc content in the tissues gradually increased as the concentration of ZnO-NPs increased, the fish exposed to the lowest concentration (6 mg/l) of ZnO-NPs recorded the lowest content of Zn while those exposed to the highest concentration (12 mg/l) of ZnO-NPs accumulated the highest content of Zn compared to control (Table 2). The content of Zn ranging from 0.002 mg/g to 0.051 mg/g recorded in serum, bone, kidney, muscle and skin of *H. longifilis* between 15 and 45 d of exposure to ZnO-NPs was found to be below the recommended acceptable limits (0.05 mg/g) of Zn in fish tissues by WHO/FAO (1989); FEPA (1991); WHO (1993) and NESREA (2011), but the recorded accumulated Zn ranging from 0.068 mg/g to 0.111mg/g in the above-mentioned tissues was above the acceptable limits after 60 d of exposure to ZnO-NPs except in the bone with value of Zn (0.054 mg/g) which was still within the acceptable limits (Table 2). However, the recorded accumulated Zn in gills, gastro-intestinal tract (GIT) and liver ranging between 0.055 mg/g and 0.263 mg/g was above the recommended acceptable limits in fish tissues from 15 to 60 d of exposure to ZnO-NPs (Table 2). Accumulation of Zn in the tissues of the ZnO-NPs-exposed fish increased with increase in the exposure period. Bio-concentration of Zn in the tissues of *H. longifilis* is therefore concentration and time dependent. At the end of 15 d of depuration, the content of accumulated Zn in all the tissues gradually decreased, with values (0.056 mg/g - 0.263 mg/g)

Table 1: Mean water quality parameters during exposure of *Heterobranchus longifilis* for 60 days and depuration period for 30 days

CONC. (mg/l)	TDS (mg/l)	DO (mg/L)	BOD (mg/L)	COD (mg/L)	Conductivity (µ S/cm)	Turbidity	TEMP (0C)	PH
	Chronic (60 d)							
0	1.20 ± 0.10 [a]	8.08 ± 1.00 [d]	4.68 ± 0.10 [a]	30.28 ± 1.00 [a]	58.21 ± 1.00[a]	1.44 ± 1.00 [a]	25.0 ± 0.20 [a]	7.0 ± 1.10 [a]
6	5.10 ± 1.00 [a]	4.4 ± 0.20 [c]	7.12 ± 1.00 [b]	36.9 ± 1.10 [b]	62.19 ± 2.00 [b]	5.14 ± 0.20 [b]	26.9 ± 0.20 [a]	7.0 ± 1.20 [a]
8	5.50 ± 1.10 [a]	3.64 ± 0.10 [b]	9.44 ± 1.20 [c]	40.2 ± 0.10 [c]	68.18 ± 0.20 [c]	5.47 ± 0.30 [b]	26.9 ± 0.10 [a]	6.5 ± 0.10 [a]
10	6.10 ± 1.20 [a]	2.87 ± 1.10 [a]	12.74 ± 1.00 [d]	48.6 ± 0.10 [d]	86.82 ± 1.10 [d]	7.31 ± 0.30 [c]	26.9 ± 1.10 [a]	6.5 ± 0.20 [a]
12	6.70 ± 0.30 [a]	2.25 ± 1.00 [a]	12.88 ± 1.00[d]	53.28 ± 0.10 [e]	118.10 ± 2.10 [e]	9.28 ± 1.00 [d]	26.9 ± 0.20[a]	6.2 ± 1.00 [a]
	Depuration (30 d)							
0	1.10 ± 1.00 [a]	8.15 ± 2.00 [c]	5.90 ± 0.20 [a]	30.24 ± 0.20 [a]	58.14 ± 2.00 [a]	1.43 ± 0.10 [a]	25.0 ± 0.10 [a]	7.0 ± 0.02 [a]
6	4.80 ± 0.10 [b]	5.12 ± 1.00 [b]	6.98 ± 0.30 [b]	32.39 ± 0.20 [a]	60.15 ± 1.00 [b]	5.04 ± 1.10 [b]	25.0 ± 0.10 [a]	7.0 ± 0.02 [a]
8	4.96 ± 0.10 [b]	4.33 ± 1.00 [a]	9.13 ± 2.00 [c]	36.21 ± 1.02 [b]	65.09 ± 1.00 [c]	5.16 ± 0.20 [b]	25.0 ± 0.10 [a]	7.0 ± 0.02 [a]
10	5.80 ± 0.02 [b]	3.45 ± 2.00[a]	11.45 ± 2.00 [d]	38.67 ± 2.00 [b]	78.31 ± 2.00 [d]	6.58 ± 2.00 [c]	24.0 ± 0.10 [a]	7.0 ± 0.02 [a]
12	6.20 ± 1.00 [c]	3.14 ± 2.00 [a]	12.02 ± 0.40 [d]	45.72 ± 1.00 [c]	112.17 ± 2.00 [e]	7.84 ± 2.00 [d]	24.0 ± 0.10 [a]	7.0 ± 0.02 [a]

Mean (± S.D., n=3) with the same superscript in the same column are not significantly different (P > 0.05)
TDS = Total dissolved solids; DO = Dissolved; Temp. = Temperature; BOD = Biochemical oxygen demand;
COD = Chemical oxygen demand.
Standard for water quality by FEPA (1991), WHO (2004) and NESTREA Limits are DO = 4-6 mg/l; BOD = 4-6 mg/l; COD = 30 mg/l; pH = 6.5-9.0; Temp. = 20-33 ^{0}C; TURB. = 4 mg/l

above the maximum recommended value of Zn in fish tissues (Table 2). However, as the depuration period increased to 30 days, the average concentration of Zn decreased in all the tissues but the value of Zn in the gills, GIT, kidney, liver and skin (0.175 mg/g, 0.162 mg/g, 0.066 mg/g, 0.071 mg/g and 0.073 mg/g respectively) still remained above the maximum permissible limits of Zn in fish tissues. However, the blood, bone and muscle were able to depurate the zinc content to values (0.034 mg/g, 0.031 mg/g and 0.044 mg/g, respectively) below the acceptable limits of Zn in fish tissues (Table 2).

Bio-Concentration Factor (BCF) of Zn in different tissues after 60 days of exposure to varying concentrations of ZnO-NPs were revealed in Figures 1 and 2. Figure 1a reveals the level of Zn in various tissues after 15 days of exposure, while Figure 1b shows the level of Zn at the end of 30 days of exposure and Figure 1c reveals Zn level at 45 d of exposure. The level of Zn gradually increased in each tissue as the exposure period increased. After the 60th day of exposure, the level of Zn that was concentrated in each tissue greatly increased and varied with the tissues (Figure 2 d). The least concentration was recorded in the bone and the highest concentration in the gills. The increasing order of concentration of Zn is bone < muscle < blood < skin < kidney < liver < GIT < gills and accumulation were time dependent (Figures 1 and 2).

After the 30th day of depuration, only bone, muscle and serum that accumulated less Zn were able to depurate to a level that is within the acceptable limits of Zn in the tissues of fish. The remaining fish tissues (skin, kidney, liver, GIT and gills) that accumulated more Zn also depurated but were still above the permissible limits of Zn in fish tissues. There was great reduction in the concentrations of Zn in all the tissues of *H. longifilis* after 30 days of depuration, but the zinc content was still higher compared to the control and was above the permissible limits in fish tissues (Figure 2e and f).

Table 2: - Bio-accumulation of zinc in different tissues of *Heterobranchus longifilis* exposed to zinc oxide nano-particles for 60 days and depurated for 30 days

	Exposure period (day)				Recovery period (days)	
Conc.(mg/l)	15	30	45	60	15	30
Blood						
0	0.001 ± 0.00^{au}	0.001 ± 0.00^{au}	0.001 ± 0.00^{au}	0.001 ± 0.00^{au}	0.001 ± 0.00^{au}	0.001 ±0.00^{au}
6	0.008 ± 0.00^{bu}	0.013 ± 0.01^{bv}	0.016 ±0.00^{bw}	0.076 ± 0.01^{bz}	0.051 ± 0.00^{by}	0.031 ± 0.01^{bx}
8	0.009 ± 0.01^{cu}	0.014 ± 0.01^{cv}	0.017 ± 0.01^{cw}	0.079 ± 0.01^{cz}	0.053 ± 0.01^{cy}	0.032 ± 0.01^{cx}
10	0.009 ± 0.00^{cu}	0.014 ± 0.01^{cv}	0.018 ± 0.01^{dw}	0.081 ± 0.01^{dz}	0.055 ± 0.01^{dy}	0.033 ± 0.02^{dx}
12	0.011 ± 0.01^{du}	0.016 ± 0.02^{dv}	0.019 ± 0.02^{ew}	0.088 ± 0.01^{ez}	0.056 ± 0.02^{ey}	0.034 ± 0.01^{ex}
Bone						
0	0.000 ± 0.00^{au}	0.000 ± 0.00^{au}	0.000 ± 0.00^{au}	0.000 ± 0.01^{au}	0.000 ± 0.00^{au}	0.000 ± 0.00^{au}
6	0.000 ± 0.00^{au}	0.004 ± 0.00^{bv}	0.009 ± 0.01^{bw}	0.041 ± 0.00^{bz}	0.040 ± 0.00^{by}	0.027 ± 0.00^{bx}
8	0.001 ± 0.01^{bu}	0.007 ± 0.01^{cv}	0.011 ± 0.02^{cw}	0.047 ± 0.02^{cz}	0.045 ± 0.01^{1y}	0.028 ± 0.01^{cx}
10	0.001 ± 0.01^{bu}	0.008 ± 0.00^{dv}	0.012 ± 0.01^{dw}	0.054 ± 0.01^{dz}	0.048 ± 0.01^{dy}	0.029 ± 0.02^{dx}
12	0.002 ± 0.01^{cu}	0.009 ± 0.00^{ev}	0.013 ± 0.02^{ew}	0.054 ± 0.02^{dz}	0.050 ± 0.02^{ey}	0.031 ± 0.01^{ex}
GIT						
0	0.002 ± 0.00^{au}	0.002 ± 0.00^{au}	0.002 ± 0.00^{au}	0.002 ± 0.00^{au}	0.002 ± 0.00^{au}	0.002 ± 0.00^{au}
6	0.064 ± 0.00^{bu}	0.073 ± 0.01^{bv}	0.142 ± 0.01^{bw}	0.253 ± 0.01^{bz}	0.166 ± 0.01^{by}	0.158 ± 0.01^{bx}
8	0.065 ± 0.01^{bu}	0.075 ± 0.01^{cv}	0.143 ± 0.01^{cw}	0.258 ± 0.02^{cz}	0.168 ± 0.02^{by}	0.159 ± 0.01^{cx}
10	0.069 ± 0.01^{cu}	0.078 ± 0.02^{dv}	0.148 ± 0.01^{dw}	0.259 ± 0.01^{dz}	0.169 ± 0.01^{cy}	0.161 ± 0.02^{dx}
12	0.071 ± 0.00^{du}	0.082 ± 0.01^{ev}	0.154 ± 0.02^{ew}	0.261 ± 0.02^{ez}	0.171 ± 0.02^{cy}	0.162 ± 0.01^{ex}
Gill						
0	0.002 ± 0.00^{au}	0.002 ± 0.00^{au}	0.002 ± 0.00^{au}	0.002 ± 0.00^{au}	0.002 ± 0.00^{au}	0.002 ± 0.00^{au}
6	0.030 ± 0.00^{bu}	0.098 ± 0.02^{bv}	0.148 ± 0.01^{bw}	0.243 ± 0.02^{bz}	0.182 ± 0.01^{by}	0.165 ± 0.01^{bx}
8	0.050 ± 0.01^{cu}	0.106 ± 0.01^{cv}	0.152 ± 0.01^{cw}	0.259 ± 0.02^{cz}	0.183 ± 0.01^{cy}	0.168 ± 0.01^{cx}
10	0.050 ± 0.01^{cu}	0.109 ± 0.01^{dv}	0.159 ± 0.02^{dw}	0.261 ± 0.02^{dz}	0.183 ± 0.01^{cy}	0.172 ± 0.01^{dx}
12	0.060 ± 0.02^{du}	0.113 ± 0.02^{ev}	0.161 ± 0.01^{ew}	0.263 ± 0.02^{ez}	0.184 ± 0.01^{dy}	0.175 ± 0.01^{ex}
Kidney						
0	0.001 ± 0.00^{au}	0.001 ± 0.00^{au}	0.001± 0.00^{au}	0.001 ± 0.00^{au}	0.001 ± 0.00^{au}	0.001 ± 0.00^{au}
6	0.007 ± 0.01^{bu}	0.011 ± 0.00^{bv}	0.018 ± 0.01^{bw}	0.098 ± 0.01^{bz}	0.056 ± 0.01^{by}	0.049 ± 0.01^{bx}
8	0.009 ± 0.01^{cu}	0.013 ± 0.01^{cv}	0.018 ± 0.01^{bw}	0.099 ± 0.01^{cz}	0.057 ± 0.01^{cy}	0.051 ± 0.01^{cx}
10	0.009 ± 0.01^{cu}	0.015 ± 0.01^{dv}	0.021 ± 0.01^{cw}	0.101 ± 0.01^{dz}	0.068 ± 0.01^{dy}	0.062 ± 0.01^{dx}
12	0.013 ± 0.01^{du}	0.016 ± 0.01^{ev}	0.022 ± 0.01^{dw}	0.111 ± 0.01^{ez}	0.071 ± 0.02^{ey}	0.066 ± 0.02^{ex}
Muscle						
0	0.002 ± 0.00^{au}	0.002 ± 0.00^{au}	0.002 ± 0.00^{au}	0.002 ± 0.00^{au}	0.002 ± 0.00^{au}	0.002 ± 0.00^{au}
6	0.004 ± 0.00^{bu}	0.011 ± 0.01^{bv}	0.013 ± 0.01^{bw}	0.059 ± 0.01^{bz}	0.049 ± 0.01^{by}	0.043 ± 0.01^{bx}
8	0.007 ± 0.01^{cu}	0.012 ± 0.01^{cv}	0.019 ± 0.01^{cw}	0.061 ± 0.01^{cz}	0.051 ± 0.01^{cy}	0.043 ± 0.01^{bx}
10	0.008 ± 0.01^{du}	0.013 ± 0.01^{dv}	0.019 ± 0.01^{dw}	0.063 ± 0.01^{dz}	0.046 ± 0.01^{dy}	0.043 ± 0.01^{bx}
12	0.009 ± 0.02^{eu}	0.014 ± 0.02^{ev}	0.021 ± 0.02^{ew}	0.068 ± 0.01^{ez}	0.056 ± 0.01^{ey}	0.044 ± 0.02^{cx}
Liver						
0	0.001 ± 0.00^{au}	0.001 ± 0.00^{au}	0.001 ± 0.00^{au}	0.001 ± 0.00^{au}	0.001 ± 0.00^{au}	0.001 ± 0.00^{au}
6	0.043 ± 0.01^{bu}	0.053 ± 0.01^{bv}	0.092 ± 0.01^{bw}	0.123 ± 0.01^{bz}	0.071 ± 0.01^{by}	0.064 ± 0.01^{bx}
8	0.046 ± 0.02^{cu}	0.058 ± 0.01^{cv}	0.096 ± 0.01^{cw}	0.124 ± 0.01^{cz}	0.072 ± 0.01^{cy}	0.065 ± 0.01^{cx}
10	0.052 ± 0.01^{du}	0.061 ± 0.01^{dv}	0.097 ± 0.01^{dw}	0.127 ± 0.01^{dz}	0.073 ± 0.01^{dy}	0.069 ± 0.01^{dx}
12	0.055 ± 0.02^{eu}	0.062 ± 0.02^{ev}	0.103 ± 0.02^{ew}	0.129 ± 0.01^{ez}	0.077 ± 0.02^{ey}	0.071 ± 0.02^{ex}
Skin						
0	0.001 ± 0.00^{au}	0.001 ± 0.00^{au}	0.001 ± 0.00^{au}	0.001 ± 0.00^{au}	0.001 ± 0.00^{au}	0.001 ± 0.00^{au}
6	0.031 ± 0.00^{bu}	0.037 ± 0.00^{bv}	0.046 ± 0.00^{bw}	0.098 ± 0.01^{bz}	0.096 ± 0.01^{by}	0.067 ± 0.01^{bx}
8	0.036 ± 0.01^{cu}	0.039 ± 0.01^{cv}	0.048 ± 0.01^{cw}	0.101 ± 0.02^{cz}	0.097 ± 0.01^{cy}	0.068 ± 0.01^{cx}
10	0.038 ± 0.01^{du}	0.042 ± 0.01^{dv}	0.049 ± 0.02^{dw}	0.103 ± 0.02^{dz}	0.099 ± 0.01^{dy}	0.069 ± 0.01^{dx}
12	0.039 ± 0.01^{eu}	0.046 ± 0.02^{ev}	0.051 ± 0.01^{ew}	0.108 ± 0.01^{ez}	0.102 ± 0.02^{ey}	0.073 ± 0.01^{dx}

Values are means ±SD (n=3). Column values with different superscripts (a-e) are significantly different ($P < 0.05$)
Row values with different superscripts (u-z) are significantly different ($P < 0.05$)

Discussion

The highest accumulation of zinc in the gill could be due to direct adsorption of Zn nano-particles on the gill filament or penetration of Zn nano-particles across the gill membrane, the first tissue that has contact with ambient water (Hao *et al.*, 2013). The role of gill in the transport and excretion of materials could also lead to significantly high levels of zinc content in the gill tissues of *H. longifilis* (De La Torre *et al*., 2007). The high accumulation of zinc reported in the gills of *H. longifilis* in this study is in contrast with the findings of Kaya *et al*. (2015), who reported the highest accumulation of zinc in the intestine of tilapia compared to other organs after exposure to ZnO-NPs. This could be a result of different adaptive characteristics in different fish species. An increase in the accumulation of Zn in the tissues of *H. longifilis* exposed to ZnO-NPs as the concentration and exposure period increased agrees with the findings of Kaya *et al.* (2015), who also reported an increase in the accumulation of ZnO-NPs in tissues of *Oreochromis niloticus* with an increase in the concentration of and exposure period. Increase in the content of zinc in the gills, intestine, liver and kidney in *H. longifilis* conforms with the findings of Kaya *et al*. (2015) involving *O. niloticus* exposed to 10 mg/l ZnO-NPs. Bioaccumulation of ZnO-NPs in the gills, intestine, liver and kidney of *H. longifilis* caused several damages that have adverse effects on the physiology of the fish (Abdulkareem & Owolabi, 2019; Owolabi & Abdulkareem, 2021). These organs could therefore serve as biomarkers for ZnO-NPs toxicity in *H*. *longifilis*. Hao *et al*. (2013) also reported high zinc accumulation in organs of carp exposed ZnO-NPs. However, the order of accumulation of zinc in *H. longifilis* in this study is gill→ gastro-intestinal tract→liver → kidney → skin → blood → muscle → bone, is in contrast with the order of ZnO-NPs accumulation in tilapia (intestine → liver →kidney → gills → brain → muscle) reported by Kaya *et al*. (2015). Muscles are among organs with lower accumulation of zinc. This result is in accordance with the reports of Zhang *et al*. (2007), Ramsden *et al*. (2009) and Hao *et al.* (2013), who reported less concentration of titanium in the muscles of *Cyprinus carpio* when exposed to titanium oxides. Kaya *et al*. (2015), who also reported lower concentration of Zn in *Orechromis niloticus;* and Ramsden *et al*. (2009), who

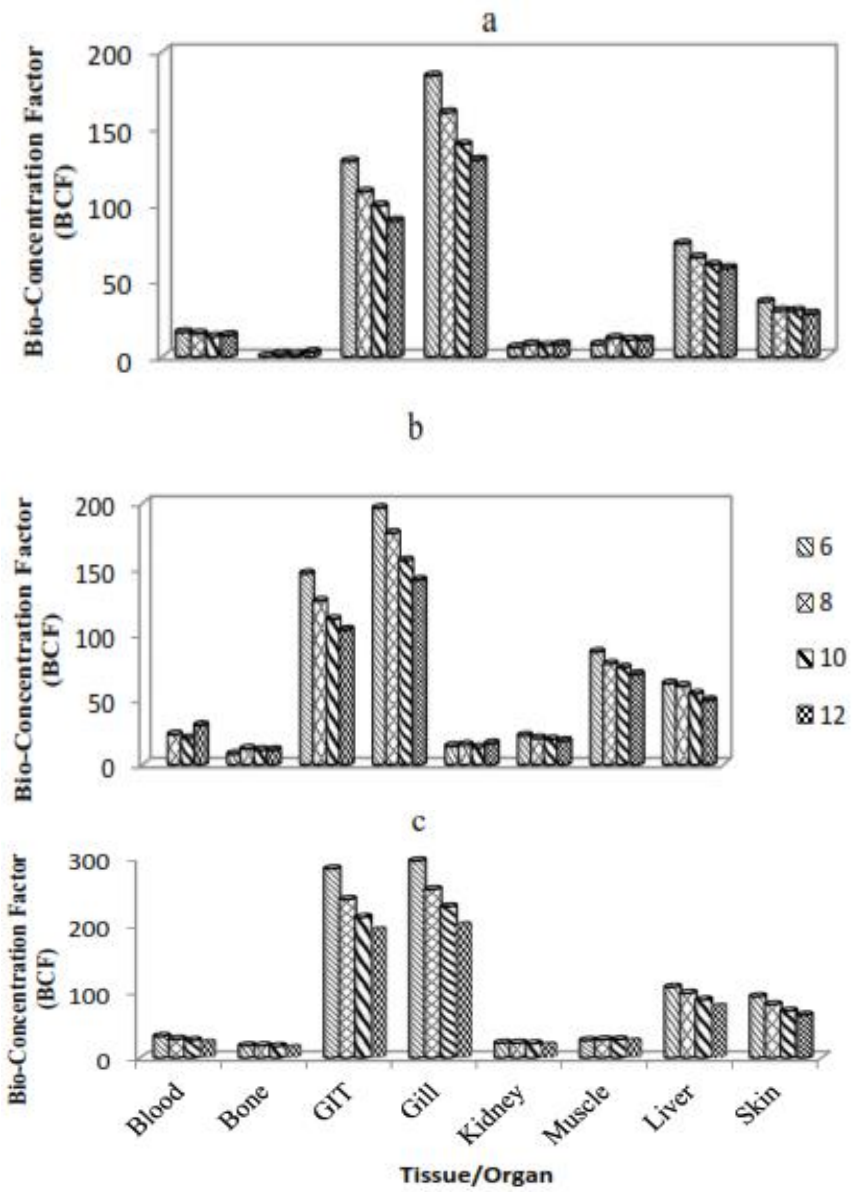

Figure 1: Bio-Concentration Factor (BCF) of Zinc in different tissues of *Heterobranchus longifilis* after a) 15, b) 30, c) 45 days of exposure to varying concentrations of ZnO-NPs

similarly reported the muscle of rainbow trout. Accumulation of zinc in the intestine could be as a result of the mucosa layer that holds metals (Clearwater *et al*., 2002). High accumulation of Zn in the liver could be attributed to the roles of liver in chemical assimilation, excretion and detoxification.

This enabled it to assimilate ingested metals, thereby accumulating in the cells (Marijic & Raspor, 2006). Another target organ for metal accumulation is the kidney. This could be because it also helps in metal excretion through the bonding of the metals to sequestering proteins such as metallothionein (MT) (Heath, 1987), which are produced in the kidney.

In conclusion, the gills accumulated the highest content of zinc while bone accumulated the lowest, but was above the maximum acceptable limits in fish tissues. The increasing order of concentration of Zn is as bone < muscle < blood < skin < kidney < liver < GIT < gills and accumulation were concentration and time-dependent. There were great reductions in the concentrations of Zn in all the tissues of *H. longifilis* after 30 days of depuration; however, the recorded concentrations were still higher compared to the control group. The fish is therefore capable of depurating the accumulated metal naturally, but the ability to depurate is time-dependent.

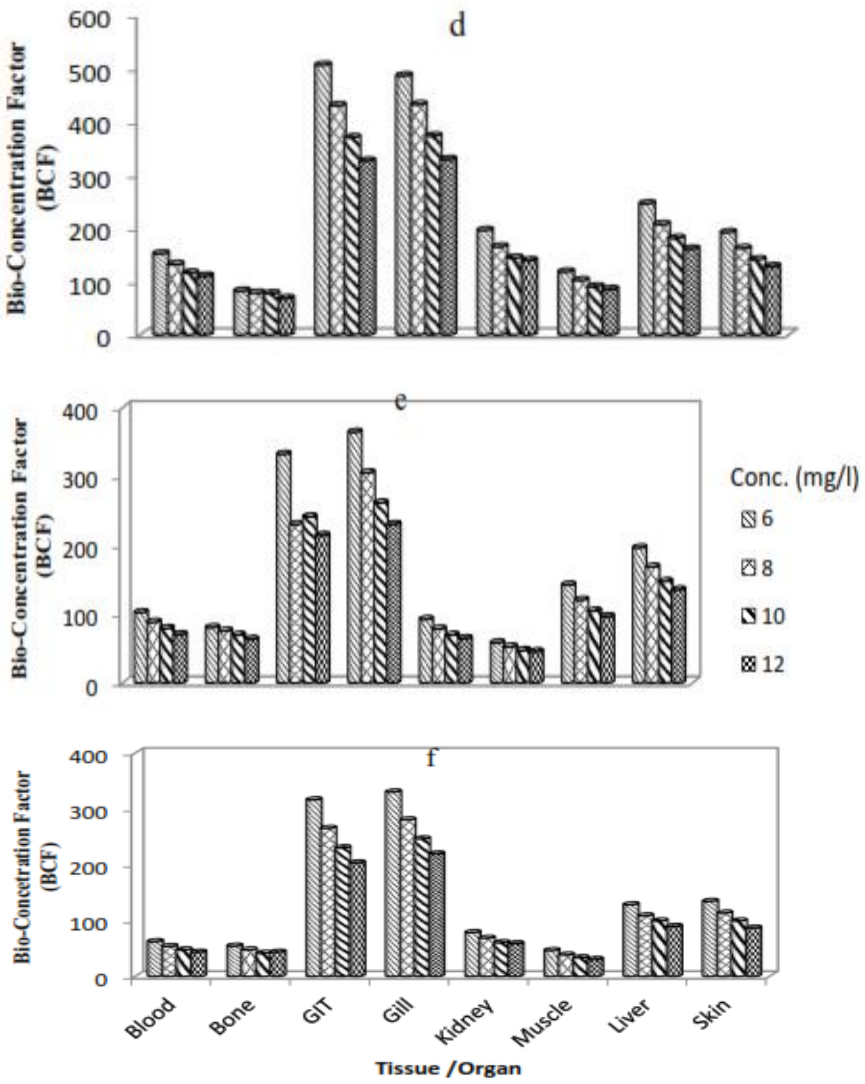

Figure 2: Bio-Concentration Factor (BCF) of Zinc in different tissues of *Heterobranchus longifilis* exposed to varying concentrations of ZnO-NPs after d) 60 days and depurated for e) 15 and f) 30 days

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Conflict of interest

The authors declare that there is no conflict of interest.

References

Abdulkareem SI & Owolabi OD (2019). Acute toxicity of zinc oxide nanoparticles on blood cell morphology, haematology and histopathology of *Heterobranchus longifilis*. *Animal Research International*, **16**(1): 3174-3185.

Adams L, Lyon DY & Alvarez PJJ (2006). Comparative ecotoxicity of nanoscales TiO2, SiO2, and

ZnO water suspensions. *Water Resources,* **40** (19): 3527–3532.

Ademoroti CMA (1996). Standard Method for Water and Effluents Analysis. Foludex press Limited*,* Ibadan, Nigeria. Pp 22-23, 44-54, 111-112.

Akanbi-Gada MA, Ogunkunle CO, Vishwakarma V, Viswanathan K & Fatoba PO (2019). Phytotoxicity of nano-zinc oxide to tomato plant (*Solanum lycopersicum* L): Zn uptake, stress enzymes response and influence on non-enzymatic antioxidants in fruits. *Environmental Technology Innovation,* doi.10.9790/2402-1310010915.

Ali MM, Ali ML Islamc MS & Rahman MZ (2016). Preliminary assessment of heavy metals in water and sediment of Karnaphuli River. *Environmental Nanotechnology, Monitoring and Management*, doi.10.1016/j.enmm.2016.01.002.

APHA (American Public Health Association) American water works Association and Water pollution control federation (1995). Standard methods for Examination of Water and waste water, nineteenth edition. *American Public Health Association,* Washington, D.C. Pp 170.

Aruoja V, Dubourguier HC, Kasemets K & Kahru A (2009). Toxicity of nanoparticles of CuO, ZnO and TiO_2 to microalgae *Pseudokirchneriella subcapitata*. *Science and Total Environment,* **407**(4): 1461–1468.

Blaise C, Gagné F & Ferard JF (2008). Ecotoxicity of selected nano-materials to aquatic organisms. *Environmental Toxicology,* **23** (5): 591–598.

Blinova I, Ivask A, Heinlaan M, Mortimer M & Kahru A (2010). Ecotoxicity of nanoparticles of CuO and ZnO in natural water. *Environmental Pollution*, **158**(1): 41-47.

Buerki-Thurnherr T, Xiao L, Diener L, Arslan O, Hirsch C & Maeder-Althaus X (2013). *In vitro* mechanistic study towards a better understanding of ZnO nanoparticle toxicity. *Nanotoxicology,* **7**(4): 402–416.

Clearwater, SJ, AM Farag & JS Meyer (2002). Bioavailability and toxicity of dietborne copper and zinc to fish. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology,* **132**(3): 269-313.

De la Torre FR, Salibián A & Ferrari L (2007). Assessment of the pollution impact on biomarkers of effect of a freshwater fish. *Chemosphere,* **68**(8): 1582–1590.

Duncan DB (1955). Multiple ranges and multiple F-tests. *Biometrics Fisheries Resources,* doi.10.2307/3001478.

Fan W, Li Q, Yang X & Zhang L (2013). Zinc subcellular distribution in liver of goldfish (*Carassius auratus*) with exposure to zinc oxide nanoparticles and mechanism of hepatic detoxification. *PLOS one,* doi.10.1371/journal.pone.0078123.

FAO (1986). Irrigation Water Management: Irrigation Water Needs. Training manual no. 3. Food and Agriculture Organization of the United Nations, *Via delle Terme di Caracalla, 00100 Rome,* Italy. FAO series 20. Pp 52.

Fawole OO, Ogundiran MA, Ayandiran TA & Olagunju OF (2007). Mineral composition in some selected fresh water fishes in Nigeria. *Journal of Food Safety*, **9**: 52-55.

FEPA (Federal Environmental Protection Agency) (1991). Guidelines and Standards for Environmental Pollution Control in Nigeria. Environmental Policy, Federal Government Printer of Lagos, Nigeria. Pp 1-238**.**

Hao L, Chen L, Hao J & Zhang N (2013). Bioaccumulation and sub-acute toxicity of zinc oxide nanoparticles in juvenile carp (*Cyprinus carpio*): A comparative study with its bulk counterparts. *Ecotoxicology and environmental safety*, doi.10.1016/j.eoeng.2013.01.007.

Heath AG (1987). Behaviour and Nervous System Function. In: *Water Pollution and Fish Physiology,* CRC Press, Boca Raton, Florida. Pp 181.

Johnston BD, Scown TM & Moger J (2010). Bioavailability of nanoscale metal oxides TiO2, CeO2, and ZnO to Fish. *Environmental Science and Technology,* **44**(3): 1144–1151.

Kaya H, Fatih A, Mert G, Sevdan Y, Mehmet A, Veysel D & Zikri A (2015). Effects of zinc oxide nanoparticles on bioaccumulation and oxidative stress in different organs of tilapia (*Oreochromis niloticus*) *Environmental Toxicology and Pharmacology*, **40**(3): 936-947.

Linhuan H, Lie C, Jianmin H & Na Z (2013). Bioaccumulation and sub-acute toxicity of Zinc oxide nanoparticles in juvenile carp (*Cyprinus carpio)*: A comparative study of with its bulk counter parts. *Ecotoxicology*

and Environmental Safety, doi.10.1016/j.eoeng.2013.01.007.

Marijic VF & Raspor B (2006). Metal exposure assessment in native fish, Mullus barbatus, from the Eastern Adriatic Sea. *Toxicology,* **168** (3):292-301.

Matinise N, Fuku XG, Kaviyarasu K Mayedwa N & Maaza M (2017). ZnO nanoparticles via *Moringa oleifera* green synthesis: Physical properties and mechanism of formation. *Applied Surface Science*, **406** (6) 339-347.

Meyer JP, Allen NJ & Smith C (1993). Commitment to Organizations and occupations: Extension and test of a three-component conceptualization, *journal of applied psychology*, **78**(4): 538-551.

NESREA (National Environmental Standards and Regulations Enforcement Agency) (2011). National Environmental (Surface and groundwater quality control) regulations. Pp 693-727.

Owolabi OD & Abdulkareem SI (2021). Effects of vitamin C on structural changes in the tissues of the giant African catfish, Heterobranchus longifilis exposed to Zinc Oxide nanoparticles. Egyptian Journal of Aquatic Biology & Fisheries*,* **25**(5): 103 – 129.

Ramsden CS, Smith TJ, Shaw BJ & Handy RD (2009). Dietary exposure to titanium dioxide nanoparticles in rainbow trout, (*Oncorhynchus mykiss*): No effect on growth, but subtle biochemical disturbances in the brain. *Ecotoxicology*, **18**(7): 939–951.

USEPA (United States Environmental Protection Agency) (1996). Ecological effects test guidelines OPPTS 850.4200 toxicity test. EPA 712 (C): 96-154. **365** (1-3): 17-42.

Uysal K (2011). Heavy metal in edible portions (muscle and skin) and other organs (gills, liver and intestine) of selected freshwater fish species. *International Journal of Food Properties*, **14**(2): 280–286.

Wang M, Cao JL & Han GJ (2011). Effect of nanometer zinc oxides on CAT activities in liver, kidney, and brain of carps. *Journal of Huaihai Institute of Technology (National Science Education,* **20** (3): 89–92.

WHO (World Health Organization) (1993). Summary of WHO guidelines for the assessment of herbal medicines. *Herbal Gram,* **28**: 13-14.

WHO (World Health Organization) (2004). Report of the seventh WHOPES working group meeting, 2–4 December 2003, *Geneva.*

WHO/FAO (1989). National Research Council Recommended Dietary 626 Allowances, tenth edition. National Academy Press. Washington, DC. USA.

Yang H, Liu C, Yang HS & Xi Z (2009). Comparative study of cytotoxicity, oxidative stress and genotoxicity induced by four typical nanomaterials: The role of particle size, shape and composition. *Journal of Applied. Toxicology*. **29** (3): 69-78.

Yi Y, Yanga Z & Zhang S (2011). Ecological risk assessment of heavy metals in sediment and human health risk assessment of heavy metals in fishes in the middle and lower reaches of the Yangtze River basin. *Environmental Pollution*. **159**(10): 2575–2585.

Zhang X, Hongwen S, Zhang Z, Niu Q, Chen Y & Crittenden JC (2007). Enhanced bioaccumulation of cadmium in carp in the presence of titanium dioxide nanoparticles. *Chemosphere,* **67**(1): 160–166.

Zimmermann YS, Schäffer A, Hugi C, Fent K, Corvini PFX & Lenz M (2012). Organic photovoltaics: potential fate and effects in the environment. *Environment International,* doi.10.1016/j.envint.2012.0.015.